MAR -8 1954 B 464207

VOLUME 12 NUMBER 3

NUTRITION REVIEWS

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March 1954

Special Article
Early Experiences with Pantothenic Acid—A Retrospect 65
Animal Nutrition
Threonine and Valine Requirement of Growing Swine 68
Nutrition and Agriculture
Improvement of Corn Proteins with Amino Acids 70
Nutrition Resources
Food Potentials in the Sea 72
Experimental Nutrition
Sodium Toxicity in Potassium Deficiency 75
Some Studies on Mineral Nutrition 77
Metabolic Influences on Tooth Development During Pregnancy ... 78
Ascorbic Acid and Adrenocortical Function 81
The Metabolism of Radioactive Niacin and Niacinamide in the Rat 82
Comparative Urinary Excretion of D- and L-Isomers of Amino Acids 84
Genetic and Environmental Factors in Growth 86
The Effect of Choline on Fatty Acid Oxidation 88
Dietary Protein and Reproduction in Rats 90
Aureomycin and Riboflavin Metabolism in Microorganisms 92
Notes
A British Opinion on Fluoridation 95
Maize and Maize Diets 96
Automatic Recording of Bacterial Growth Rates 96

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

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Changes of address should be sent to The Nutrition Foundation.
Application for entry as second-class matter pending at the Post Office at Baltimore, Md.

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NUTRITION REVIEWS

VOL. 12 MARCH 1954 No. 3

EARLY EXPERIENCES WITH PANTOTHENIC ACID—A RETROSPECT

My earliest experience with the biologic effect of pantothenic acid came when as a graduate student at the University of Chicago in the summer of 1918 I began watching the behavior of single cells of "Old Process" yeast in hanging droplets, when suspended in various culture media. I had asked Dr. R. E. Lee, a Harvard medical man who had never practiced medicine but had gone into the yeast business instead, what was the smallest amount of yeast I could culture in pursuing my objectives under a Fleischmann Fellowship to study yeast nutrition. He suggested the possible use of hanging droplets, such as were used in isolating pure cultures, for the study of single cells.

I remember clearly the first single yeast cell I isolated. I was to isolate thousands in my later work, but none made the impression that this one did, because it was then a chore to isolate it and its behavior was unexpected. It was a perfectly good looking, plump but not overly obese yeast cell and was suspended in a synthetic medium in which the yeast en masse seemed to grow with appreciable enthusiasm. Once I had it in focus under the microscope, I watched it hour by hour. Nothing happened! I concluded that it was a slow starter. I found a means of spotting it on the slide and put it in an incubator overnight, expecting the next day to find a cluster of yeast cells. The next day it was as quiescent (and remained so for the several days that I watched it) as if it had been put to sleep. As I subsequently watched other yeast cells in similar media, I found that some single cells would grow a little and some wouldn't at all, but that they *all* would grow to clusters like bunches of grapes if even a little decoction of yeast was added to the culture medium. If a substantial amount of decoction was added, the hanging droplets would be filled with thousands of yeast cells.

The conclusion which was arrived at after my thesis study was completed was that the stimulating substance appeared to be "vitamin B," a hypothetic substance which at that time was very poorly characterized and eventually, of course, was found to be multiple in nature. While several of the B-vitamins no doubt contributed somewhat to the growth of old process yeast under the conditions I used, the one outstanding constituent which made the greatest difference was pantothenic acid. It alone, if it had been available, would have constituted an important answer to the question: What does the synthetic medium lack? If thiamine also had been added, the results would have been even more impressive.

My experience with hanging droplet cultures had a marked effect on my future research interests. The old saying "seeing is believing" applied, and the fact that I *saw* yeast cells growing when given an unknown, and failing to grow without it, put the question dramatically before me, "What do the cells need?" It was many years and thousands of experiments later before a reasonably clear answer could be given to the question. The fact that this unknown something was found in milk suggested its nutritional importance and its presence in grains, animal tissues, etc., convinced me that it was something of general biologic importance. This made the search more compelling. We eventually worked with a strain of yeast which under our conditions would respond well to pantothenic acid as the only addendum. Using this strain of

yeast as a test organism, we eventually isolated the substance in substantially pure form.

My experience with hanging droplet cultures and with different strains of yeast (which often looked remarkably alike) gave me direct visual proof that individual cells show variation and particularly that different strains of yeast can respond wholly differently from one another. I suspect that these observations were a forerunner of my present interest in biochemical individuality and of my distress when there is an overabundant use of such expressions as *the* cell, *the* protein molecule, *the* rat, or even *the* alcoholic. Whenever we use such an expression we *imply* (which is far more dangerous than asserting) that all the things included in the designation are substantially alike and that to know one is to know all. This is not in keeping with the inductive scientific method which makes its observations first and its generalizations later.

In the early history of pantothenic acid and other yeast nutrilites a tremendous number of man-hours of work could have been saved if investigators had not *taken for granted* that yeast is yeast and is bound to behave uniformly. In my opinion the advance of our knowledge of rat nutrition has been hampered by our taking for granted that there is such a thing as *the* rat. In experiments in my laboratory involving individual rats we have found them to differ markedly from one another just as do different strains of yeast. Only on the elementary and superficial level are they all alike. They all have four legs and a tail, etc., but when it comes to nutritional requirements, quantitatively speaking, they are not the same.

My microscopic experiences brought to light a personal deficiency which has had a most marked effect on my life activity, scientific and otherwise. I never had been able to use my eyes freely for close work like many of my fellow students, but when the microscopic work entered the picture, I began to feel a serious pinch. Every expedient known was used in the course of the next twenty years to circumvent the difficulty: extensive courses of exercise, the wearing of prism corrections, surgery on the eye muscles—all without any substantial gain. I still couldn't use my eyes like other people could. During one period of a few years I did all my reading with one eye at a time using a blinder over the other eye. Even so a half hour a day of reading was about all I could stand. I found while doing a sorting job a few days ago a pair of pin hole spectacles which I designed and had made in an attempt to circumvent the difficulty. I was not sure but that by using an extra strong light these might work. They actually did, but not well enough. I even considered learning Braille, but gave it up when I realized how limited the available reading matter would be.

My principal trouble, as discovered and in part corrected twenty-two years after my first serious difficulty, was a relatively severe case of aniseikonia, an eye condition discovered a few years before through the outstanding work of Professor A. Ames of the Dartmouth Eye Institute. It affects people who, like myself, have perhaps unusually keen vision. In passing I should like to voice a serious complaint with respect to Aldous Huxley's "The Art of Seeing" (*Harpers, New York (1942)*). He blandly assumes without even suggesting another alternative that the somewhat bizarre expedients which allegedly helped his eyes would help anyone's eyes!

I have used up some space to discuss this eye difficulty because I believe it has been a blessing in disguise. Because of my inability to read extensively (especially up to twelve years ago), I have been forced to *think* long and hard. Being inadequately indoctrinated in the lore of nutrition, of medicine, of the social sciences, I believe I have been able to make some substantial

contributions in the way of new approaches in these fields. The fact that I have some peculiarities of my own, of which eyesight is one, has made it possible for me to see that possibly other people are entitled to some peculiarities of their own and to realize that peculiarities in general may be extremely important in the general scheme of things.

Those who are relatively late comers in the field of biochemistry will be interested to know that there was a time when the nutrition of microorganisms was not considered a fit subject for a biochemist to study. In those days a proposed publication dealing with yeast nutrition was all but taboo in some of our best journals. This was not wholly unjustified because there were contradictions and serious confusion—a confusion which arose largely out of the taking for granted that *the* yeast cell must always behave thus and so. Incidentally, there are too many who still use the term "yeast extract" as though it were a definitive expression, which it is not.

Those young biochemists who have ideas about some unconventional line of research may be heartened by the fact that when I was plugging away trying to make some progress on the pantothenic acid problem, I was seriously advised by a man (now deceased) whose name is known to all biochemists, that the problem did not appear to be a very significant or promising one. Needless to say, I did not heed his implied advice; in fact, it spurred me on to greater effort. In retrospect it is my opinion that without the microbiologic approach, pantothenic acid would still, in 1954, be an unknown substance.

One of the striking features of our investigation of pantothenic acid was the use of fractional electrical transport by which means we were able to obtain evidence indicating that this growth substance was present in all kinds of living things. Only a combination of the use of this tool with a microbiologic approach could have accomplished this. Perhaps even more distinctive was the fact that long before we had a sample of pantothenic acid even remotely approaching purity, we knew its approximate molecular weight, its ionization constant, that it possessed more than one hydroxyl group, a nitrogen atom with barely detectable basic properties, that it lacked aldehyde, ketone, sulfhydryl, basic nitrogen, aromatic, or sugar groups. All this was possible because we had an excellent quantitative microbiologic test, and used extensively the tool of fractional electrical transport (R. J. Williams, *J. Biol. Chem.* **110,** *589 (1935)*).

One other quite different insight arose out of my earlier and later experiences with pantothenic acid. It was the rather gradual discovery that investigators (including myself) are not the disinterested, truth-seeking machines they are sometimes thought to be, but instead are very human. When, for example, Wildiers published his paper on "bios" in 1901, neither he nor his professor (Ide, who was actually responsible for the investigation; *see* R. J. Williams, *Science* **88,** *475 (1938)*) had conspicuous scientific standing, and it was pathetic how the "bigwigs" in the field of the chemistry of brewing discredited and ignored the outstanding report. Fantastic rebuttals were indulged in and a few years later important reviews on yeast nutrition made no mention of the work. No doubt there are exceptions, but in general investigators tend to examine and adjudicate their own contributions on the basis of how they look through a magnifying glass, and to view the contributions of their competitors as though looking backwards through binoculars.

Since investigators *are* human, I'm sure there is no reason why we should expect them to be otherwise. The late George Zipf of Harvard in his remarkable book, "Human Behavior and the Principle of Least Effort" (*Addison-Wesley Press, Inc., Cambridge, Mass. (1949)*), suggested that

we would gain insight into human behavior if we viewed it purely as a natural phenomenon like everything else in the universe and if we studied it with the same dispassionate objectivity which we exhibit when we study the social behavior of bees or the nest building habits of birds.

When we follow his suggestion, at the same time taking into account *tremendous human variability*, we can develop a frame of mind toward ourselves and our "companions in zealous research," which rids us of much impatience, frustration and discomfort.

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THREONINE AND VALINE REQUIREMENT OF GROWING SWINE

For several years workers at the Purdue Agricultural Experiment Station have been engaged in studies to establish the amino acid requirement of weanling pigs (*Nutrition Reviews* **9**, *220* (*1951*)). This group demonstrated that swine could be grown on purified diets containing an amino acid mixture as the source of protein (D. C. Shelton, W. M. Beeson, and E. T. Mertz, *Arch. Biochem.* **29**, *446* (*1950*)). Using the ten amino acids essential for rat growth, these workers were able to demonstrate the essential nature of threonine for swine growth.

More recently the quantitative aspects of this problem have been reported by these workers (W. M. Beeson, H. D. Jackson, and E. T. Mertz, *J. Animal Sci.* **12**, *870* (*1953*)). The threonine quantitation was carried out using a new approach in diet formulation. Corn served as the major basic constituent of the diet and from it a balanced adequate protein ration was built up by supplementing with the ten essential amino acids and diammonium citrate.

The basal diet had the following percentage composition: corn (8.2 per cent crude protein), 88; essential amino acids, 6.04; diammonium citrate, 0.95; minerals, 4.30; liver extract, 0.50; and cerelose, 0.21. The diet was further supplemented with vitamins, including vitamin B_{12}, and with terramycin (10 mg. per pound of feed).

This diet had a total crude protein equivalent of 12.8 per cent and a digestible crude protein equivalent of 11.2 per cent. In formulating the amino acid mixture it was assumed that the D-forms of only methionine, phenylalanine, and tryptophan were physiologically active; this assumption was based on rat studies by W. C. Rose, L. C. Smith, M. Womack, and M. Shane (*J. Biol. Chem.* **181**, *307* (*1949*)). The basal diet contained 0.2 per cent of utilizable threonine. Supplements of 0.4, 0.6, 0.8, and 1.0 per cent of DL-threonine which were tested resulted in utilizable dietary levels of threonine of 0.2, 0.4, 0.5, 0.6, and 0.7 per cent, respectively.

The experimental subjects were weanling pigs (3 per treatment) with an average initial weight of 24.3 pounds. They were fed the test diets for a period of thirty-five days. Optimum growth was obtained when the diet contained 0.4 per cent of utilizable threonine (digestible L-threonine) or levels higher than this. For example, the average daily gain on the 0.2 per cent level of utilizable threonine was about 0.5 pounds per pig daily, whereas 0.4 per cent or more of threonine resulted in daily gains of about 1.0 pound daily. This is considered to be a satisfactory growth rate for pigs of this age and weight.

Companion studies with the same diet were also conducted with rats, with essentially the same results, *i.e.*, optimum weight gain was obtained when the diet

contained 0.4 per cent or more of utilizable threonine. This is a little lower than the threonine requirement of 0.5 per cent of the diet as reported by W. C. Rose, R. E. Koeppe, and H. J. Sallach (*J. Biol. Chem.* **194,** *317 (1952)*).

In another paper the Purdue workers have reported similar studies in which the quantitative aspects of valine were studied (H. D. Jackson, E. T. Mertz, and W. M. Beeson, *J. Nutrition* **51,** *109 (1953)*). The corn-basal diet was again used with slight modification in that adequate threonine levels were supplied while the utilizable valine levels in the diet were 0.3 (basal level), 0.4, 0.5, 0.6, and 0.7 per cent, respectively, supplied in the form of crystalline DL-valine. Optimum growth (approximately one pound per pig daily) was obtained with the diet containing 0.4 per cent of utilizable valine. The 0.3 per cent level resulted in an average daily weight gain of 0.67 pounds. Companion studies with growing rats with the same diet indicated that this species required 0.6 per cent of utilizable valine in the diet, a value in close agreement with that reported by W. C. Rose (*Science* **86,** *298 (1937)*).

Both of the Purdue experiments demonstrate that the corn protein supplemented with adequate levels of the essential amino acids and diammonium citrate resulted in remarkably high protein efficiency values. For example, in the valine study, pigs receiving 0.4 per cent of utilizable valine gained 3.2 to 3.4 pounds of body weight per pound of crude protein consumed. In the threonine experiment these values were 2.8 to 3.1 pounds of gain per pound of crude protein consumed.

The satisfactory rate of gain on a diet containing only 13.2 per cent of crude protein equivalent illustrates the excellence of corn protein for animals if it is properly balanced with respect to amino acids. The level of dietary protein that is generally recommended at present for the adequate nutrition of weanling pigs is about 18 per cent. The Purdue results indicate that this recommended protein level can be reduced if all of the essential amino acids are present in proper amounts. It should be emphasized, however, that in practical swine feeding it will generally be necessary to maintain higher levels of protein in weanling pig feeds. At present, it is uneconomical to supplement natural feeds with ideal levels of the amino acids that are essential for swine growth.

The high protein efficiency observed by the Purdue workers in pigs receiving the amino acid-supplemented corn ration is considerably higher than the values (2.1 to 2.6 pounds of gain per pound of protein consumed) recently reported for high quality natural proteins in pig feeds (H. B. Geurin, J. A. Hoefer, and W. M. Beeson, *J. Animal Sci.* **10,** *607 (1951)*; D. V. Catron *et al.*, *Ibid.* **11,** *221 (1952)*).

From presently available data the quantitative requirement of weanling pigs for four of the ten amino acids can be summarized as follows (percentage level in a diet containing 22 to 24 per cent of total protein): isoleucine, 0.7; tryptophan, 0.2; lysine, 1.0 to 1.2; and methionine, 0.6 in the absence of cystine and 0.3 in the presence of 0.3 per cent of cystine. Based on the recent Purdue experiments, the valine and threonine values are 0.4 per cent each in a diet containing 13.2 per cent of protein. It should be emphasized that the level of protein in the diet cannot be ignored in experiments of this type. For example, the above value of 1.0 to 1.2 per cent for lysine in diets containing 22 to 24 per cent of protein was decreased to 0.6 per cent when the diet contained only 10.6 per cent of protein.

The Purdue workers point out that the digestibility of protein must also be given consideration. They believe that the expression of the requirement of an amino acid in terms of the total crude protein of the diet appears to be valid only if the protein is highly digestible and well-balanced with respect to its amino acids.

IMPROVEMENT OF CORN PROTEINS WITH AMINO ACIDS

The proteins in corn have been shown to be deficient in lysine and tryptophan, while wheat proteins are deficient in lysine (H. H. Mitchell and D. B. Smuts, *J. Biol. Chem.* **95,** *263 (1932)*). Later findings of L. J. Pecora and J. M. Hundley (*J. Nutrition* **44,** *101 (1951)*) showed that the proteins of polished rice could be improved by the addition of threonine in the presence of lysine and tryptophan. B. Sure (*Arch. Biochem. Biophys.* **39,** *463 (1952)*) reported that the proteins in whole wheat and in patent whole wheat flour could be further improved by threonine in the presence of lysine and tryptophan. These findings stimulated the study of the improvement of corn proteins with these amino acids by Sure (*J. Agr. Food Chem.* **1,** *626 (1953)*).

A representative sample of whole yellow United States corn and two samples of typical Guatemalan varieties of yellow corn were tested in the rations fed fourteen groups of Wistar rats. The protein content (nitrogen × 6.25) of the United States whole corn was 8.9 per cent and of the two Guatemalan varieties was 8.3 per cent and 9.1 per cent respectively. Each group of rats contained 6 males and 6 females; they were 28 days old and each weighed 50 to 55 g. at the beginning of the experiment. The basal ration consisted of 86 per cent corn, 4 per cent mineral salts, 3 per cent vegetable shortening, 2 per cent cod liver oil, 1 per cent wheat germ oil and 4 per cent cerelose. The cerelose content was decreased in the various rations by the amount the ration was supplemented with lysine, tryptophan, methionine and threonine which were added to the ration singly or in combinations. Vitamin B-complex supplements were administered daily. Ten of the groups were fed United States whole yellow corn and four groups were fed the two Guatemalan varieties of corn. Since these Guatemalan varieties of corn contained higher concentrations of protein than the United States variety, the level of corn in the rations containing the Guatemalan varieties was reduced sufficiently that the protein content of all diets was identical.

The animals were weighed once a week and accurate records were kept of food consumption. The comparisons for protein efficiency were made by the use of the gain in body weight per gram of protein intake which was termed the protein efficiency ratio. In order to take into account the contributions toward the over-all protein requirement made by the amino acids, another index was used: the gain in body weight per gram of nitrogen intake. The addition of 1 per cent DL-lysine to the basal ration resulted in an 81 per cent increase in growth, but since the food consumption had increased 51 per cent the increase in protein efficiency ratio was only 19 per cent. The addition of 0.5 per cent DL-tryptophan in the presence of 1 per cent DL-lysine produced a total of 138 per cent increase in growth and a 66 per cent increase in protein efficiency ratio. Thus the ingestion of tryptophan produced a marked increase in the economy of food utilization. While the addition of either 0.5 per cent DL-methionine or 0.5 per cent DL-threonine resulted in negative responses, the incorporation of 0.5 per cent DL-threonine in the presence of 0.5 per cent DL-methionine, 1.0 per cent DL-lysine and 0.5 per cent DL-tryptophan resulted in a further 50 per cent increase in growth but an insignificant increase in protein efficiency ratio because of the much greater food consumption. The gains in body weight per gram of nitrogen intake increased percentage-wise almost exactly at the same rate as the protein efficiency ratio.

Using the technic of controlled food intake, further evidence was presented regarding the improvement of both the United States whole yellow corn and the two Guatemalan varieties by threonine in the presence of lysine, tryptophan and methio-

nine. In this experiment, each animal of the six groups fed the United States corn diet with various amino acid supplements was given 7 g. of the appropriate ration for fifteen days, and the food was consumed without any waste. The basal diet supplemented with 0.4 per cent DL-lysine and 0.5 per cent DL-tryptophan permitted an average of 17.0 g. gain in body weight. The addition of 0.5 per cent DL-threonine to the ration containing lysine and tryptophan did not produce a significant gain in body weight; when this threonine was replaced by 0.5 per cent DL-methionine on controlled intake, there was a slight inhibitory effect on growth. However, the addition of 0.5 per cent DL-threonine in the presence of methionine, lysine, and tryptophan was followed by about a 33 per cent increased growth and a similar increase in protein efficiency ratio, because of the greater economy in food utilization produced by the threonine addition.

The same animals were then allowed to eat the same rations for thirty days ad libitum. After the fifteen days of previous caloric restriction on the low food intake of only 7 g. daily, the threonine supplementation resulted in about 33 per cent greater increase in growth and 22 per cent greater protein efficiency ratio than in the group without threonine. Using the Mitchell method (H. H. Mitchell, *J. Biol. Chem.* **58,** *905 (1924)*) of determining nitrogen retention, the addition of 0.5 per cent DL-threonine to the ration containing United States corn plus lysine, tryptophan, and methionine resulted in an increase of 7.4 in biologic value (expressed as per cent of absorbed nitrogen retained in the animal body) and an increase of 6.0 in net utilization.

When the two types of Guatemalan corn were used in the rations, contrary to experience with the United States hybrid corn, the effect of threonine on increased growth was obtained in the absence of methionine. In this series of restricted intake experiments, the animals were placed on 7.2 per cent protein rations furnished by each of the two Guatemalan varieties, and were given 7 g. daily for thirty days. On the first variety, there was a 27 per cent increase in protein efficiency ratio as a result of the threonine addition, while on the second variety there was a 72 per cent increase in this ratio. When the same animals were fed the same rations ad libitum, the threonine addition resulted in a 54 per cent increase in growth and 31 per cent increase in protein efficiency ratio in the first variety, and a 44 per cent increase in growth and a 19 per cent increase in protein efficiency ratio in the second variety. Such results indicate the significant role which threonine plays in the economy of food utilization, which of course also influenced the protein utilization.

A biologic assay of the protein efficiency of the United States hybrid corn and the two Guatemalan varieties disclosed the following protein efficiency ratios: United States hybrid corn, 1.13; first Guatemalan variety, 1.09; second Guatemalan variety 1.09. The addition of threonine to the Guatemalan corn brought increased growth in the presence of lysine and tryptophan, but in the United States corn this response to threonine was obtained only when methionine was also added to the lysine and tryptophan. Microbiologic assays were conducted to determine the methionine content of the three types of corn used and indicated values between 0.22 and 0.23 per cent for all three varieties. It would appear then that the methionine in the U. S. variety is not readily available for digestion; hence, its addition was necessary before a response to threonine could be obtained.

At present the cost of these amino acids is sufficiently high that it is completely unrealistic to enrich corn meal for human consumption with any combination of these essential amino acids even though the desirability of such a procedure is obvious. However, the time may not be far distant

when chemically synthesized amino acids will be available in sufficiently large quantities and at inexpensive enough cost to merit utilization in an enrichment program. Already a great deal of progress has been made in the vitamin enrichment of corn in the southern United States. Progress in this public health endeavor has been described recently by E. J. Lease (*J. Am. Dietet. Assn.* **29,** *866 (1953)*). In 1946, hearings were held in Washington, D. C., on standards for corn meal enrichment with thiamine, niacin, iron, riboflavin and calcium. These Federal standards govern foods shipped from state to state, but the individual states which have enacted corn meal enrichment laws have included these standards. In South Carolina enrichment of all types of corn meal and grits that are sold has been mandatory for several years. The South Carolina Experiment Station at Clemson Agricultural College compounds an enrichment mixture for whole corn meal and distributes it to small corn mills in the south. This college also has designed and is manufacturing and distributing enrichment equipment suitable for such mills. These services are rendered on a nonprofit basis and are coordinated with an educational program to encourage millers to enrich their corn meal and grits.

It is doubtful if the average public health nutritionist or nutrition investigator in the United States realizes the extent to which corn is depended upon in various communities. For example, Sure (*loc. cit.*) stated that about 70 per cent of the proteins in Guatemala are supplied from corn. Though the intake may never reach this level in the southern United States, a detailed study of the diets of 429 farm operator families which used whole ground corn meal has shown that the corn intake is quite high (D. Dickins and O. A. Sheets, *J. Am. Dietet. Assn.* **29,** *873 (1953)*). Such an analysis was undertaken to show whether an educational program for enrichment of whole ground corn was justified for this and similar groups in the south. Eighty-four per cent of these farm operator families used whole ground corn meal at the average rate of 2.4 pounds per capita per week and simultaneously consumed 2.0 pounds of flour per capita per week. Forty-eight per cent of the families purchased their whole ground corn meal. Essentially no degerminated corn meal or grits was used. Sixteen per cent of the families did not use any corn during the study, but 31 of these 68 families used home-ground corn when corn was available for grinding. Enrichment of the whole ground corn meal would result in more families of low social economic status in this study obtaining the minimal supplies of the nutrients recommended by the National Research Council.

These studies indicate with considerable force another vast area in which the chemical industry and food manufacturers together can play a major role in the improvement of human health through the synthesis and incorporation of essential nutrients into common foods. One of the major reasons for the ultimate success of these measures is the fact that there is no necessity to change, through education or force, the food habits of individuals which often are so irrevocably dictated by custom, agricultural production potentialities and economic circumstances.

FOOD POTENTIALS IN THE SEA

At the present time, fish and shellfish are believed to contribute about 2 per cent of the total food consumption of the world's human population. Of this amount, approximately 90 per cent is taken from the waters of the northern hemisphere, while the far larger ocean expanses of the southern hemisphere have been essentially un-

touched. Because of the vast amount of nutritious food which is available in the oceans of the world and the potentially higher photosynthetic productivity of these areas than land acreages, F. J. Weiss (*J. Agr. Food Chem.* **1**, *822* (*1953*)) has presented an impressive series of illustrations of types of highly nutritious foodstuffs which could be harvested from the sea in large quantities.

Since more than 71 per cent of the earth's surface is water covered, and an acre of sea is estimated to produce nearly three times as much as an acre of land, these relatively untapped food reserves deserve careful research to determine the best means for their utilization. It has been estimated that the total photosynthetic activity of the sea is approximately ten times that of all wild and cultivated plants on land. Some 13.5×10^{10} tons of carbon are believed to be fixed per year as a result of marine photosynthesis. In addition, marine harvests require no labor or expense for tilling, irrigation, or fertilization. They are produced in a complete nutrient solution which contains not only all the essential mineral elements leached from the land masses through the centuries, but also large quantities of dissolved gases—the oceanic content of carbon dioxide has been estimated at a million times that of the earth's atmosphere. Despite these facts, this vast harvest of oceanic food has received little research to evaluate its potential role in world feeding.

Some of the other products of the sea than fish and shellfish have been used as foods or to increase the production of land crops. Chief among these are the large seaweeds belonging to the brown, green and red algae, with their great capacity to accumulate important nutrients, which have found wide use as fertilizers and soil-conditioners. Due to their high mineral content, they have been used from very early times as manure to replenish the depleted coastal soils. In addition, as early as 1911, J. MacDonald ("Agriculture of the Hebrides," *Edinburgh, Scotland* (*1911*)) reported that seaweed algin could consolidate and improve sandy soils to produce great increases in plant growth. Later it was shown that the main organic constituent of seaweed, a polymer of D-mannuronic acid, was very similar in structure and function to synthetic soil conditioners obtained by the hydrolysis of polyacrylonitrile. The use of seaweed as an animal food is a very old practice. Although it cannot be considered a complete animal food it is an excellent supplementary food and is now produced as such on the Pacific Coast. The Chinese and Japanese eat seaweed, thereby obtaining a fine source of minerals, vitamin A, and ascorbic acid. The water-soluble polysaccharides of the larger brown algae are used increasingly as stabilizers in food industries. Algin, for instance, has been used to replace gelatin as a stabilizer in ice cream and chocolate milk.

The natives on some Pacific islands successfully use the fronds of large seaweeds to protect their freshly caught fish from rapid decomposition. The recent discovery of the antibiotic action of algal tissues explains this protection. The living algae contain powerful antibiotic substances to protect them from the myriad of bacteria constantly attacking them. Further research is needed to determine if the presence of the antibiotic substances in seaweeds, rather than their high mineral content, makes them such valuable supplementary foods for cattle. Of all the various applications of seaweed and its components in the food industry, it is interesting to note that technologists in Germany have found a method to use Norwegian seaweeds in the production of an edible sausage casing, which is in many ways preferable to the cellophane skin and weighs one-twenty-ninth as much.

These larger marine algae or seaweeds which have been used as foods, fertilizers, or food components constitute only 1 per cent of the total marine vegetation. The greater

part of sea-grown plant life consists of microscopic phytoplankton (mainly diatoms and dinoflagellates). These exhibit real photosynthetic efficiency and some species are able to fix nitrogen; they contain all the major food groups—protein, carbohydrate, fat and mineral. The food chain that leads from these microscopic plants through the zooplankton, the crustaceans, through approximately two sizes of small fish to the large marine fish, such as the cod, is a long and wasteful one, for the food value from the original plant is diminished by approximately nine-tenths at each eating. So when a fisherman catches a codfish, each pound of its flesh has been calculated to represent about 100,000 pounds of plants that grew in the sea. Weiss points out the appalling wastefulness of the process and believes that this waste indicates the need for a more efficient use of the lower forms of marine life instead of dependence on the long chain from plankton to fish. In 1941, during the German submarine campaign, Sir John Graham Kerr, member of the British Parliament, asked for the appointment of a committee to investigate the large-scale collection of plankton by mechanical separation and its use as a food.

A. C. Hardy (*Nature* **147,** *707 (1941)*) pointed out the very high food value of plankton for mammals as shown by the rapid growth in whales which feed on it. The dry substance of zooplankton has an average composition of 59 per cent protein, 7 per cent fat, 20 per cent carbohydrate, 4.7 per cent chitin and 9.4 per cent ash. Its flavor is delicately shrimp-like and suitable for human food. Its relative dilution must be considered in commercial use, but if the whales can individually overcome this problem with their filter apparatus, man should be able to do so commercially. Two fishermen with suitable nets could collect off the coast of Scotland 600 pounds of plankton per day, which is estimated to contain sufficient nutrients to nourish 350 people. Some plankton organisms accumulate harmful metabolic products in their bodies that would make them undesirable for direct consumption, but extraction methods could be developed either to destroy or to separate these harmful constituents from the edible portion. Weiss recommended the use of plankton as a foodstuff supplement for animals, especially in crowded areas of the world lacking the land resources for animal production.

Some of these small crustacea feed together in a vast layer near the surface of the ocean at night and retire to a lower level during the day. This diurnal migration of an enormous mass of living creatures, which can be spotted by echo-sounding devices, was discovered by the U. S. Navy during World War II in its study of oceanic sounding devices. The ocean life of this stratum is highly nutritious and is closely related to shrimp. Special vessels could drop flexible hoses into this stratum and suck up enormous quantities of these organisms. Such collections of small animals from the ocean could be greatly facilitated by the described German commercial electrical fishing device which operates on the principle that animals when exposed to an electrical field swim to the anode and are paralyzed by sufficiently strong currents.

The culture of mussels along the French coast dates back to the thirteenth century. Acre for acre a mussel farm produces twenty to thirty times as much food as the best pasture land. The French Ministry of Fisheries reported a figure of 10,000 pounds of mussel meat per acre per year as compared with 190 pounds of beef per acre obtainable from the finest pasture (T. S. Douglas, "The Wealth of the Sea," *John Gifford, Ltd., London (1946)*). A method has been developed in Denmark to grind mussels dried in the shell at high temperature and the meal thus produced for a supplementary food for animals gave the following analysis: 71.4 per cent calcium salts, 13.2 per cent carbohydrate, 11.6 per cent protein, 1.7 per cent fat and 2.1 per cent water.

The "waste products" of the fishing

industry contain nutrients such as proteins and vitamins that in some instances are of higher food value than the portion used directly. The fish liver oils are valued in their treatment of deficiencies of the fat-soluble vitamins. "Fish flour," now manufactured in Norway from whole fish and fish waste, is an odorless, tasteless free-flowing powder high in protein and vitamins, especially the B-complex. The use of this fish flour was recommended by the United Nations Health Organization, in areas where "kwashiorkor" or malignant malnutrition was prevalent. This usually fatal disease is particularily rampant among children of those African tribes whose main dietary component is manioc, a starchy plant product, while coastal tribes which consume small amounts of fish, molluscs and crustacea are free from this disease. Malignant malnutrition is also prevalent in some rice-eating populations. Following research on this problem, the diet of children in these stricken areas was supplemented with the fish flour; already some success has been achieved in the prevention and treatment of this disease.

Weiss pointed out that one of the main drawbacks to the utilization of the nutritious marine crop is the fact that it is a perishable one and that much research is needed in marine chemistry to develop the most desirable methods for preservation and distribution of these food products. There is no question that, with additional information of this type, many more foodstuffs and other productive nutrients could be extracted from the ocean than we presently have access to. It seems unlikely that there ever would arise a circumstance in which a sufficient amount of any one of these foodstuffs could be taken from the waters of the earth to produce any detectable decrease in the marine population.

SODIUM TOXICITY IN POTASSIUM DEFICIENCY

Substantial evidence for the reciprocal role of potassium and sodium in the body has been accumulated from clinical, biochemical and pathologic studies (T. S. Danowski, *Am. J. Med.* **10,** *468 (1951)*; J. E. French, *Arch. Path.* **53,** *485 (1952)*), and it has become apparent that the manifestations of the potassium deficiency syndrome are related to the presence of relatively large amounts of sodium as much as they are to potassium lack. Previous evidence of this relationship has been reported by P. R. Cannon, L. E. Frazier, and R. H. Hughes (*Metabolism* **1,** *49 (1952)*) and more recent studies by these workers (*Ibid.* **2,** *297 (1953)*) reveal further evidence that sodium functions as a "toxic" ion in the presence of potassium deficiency.

Rats were first made protein- and, thus, potassium-deficient by the administration of low protein diets until they had a 30 to 35 per cent weight loss. The animals were then followed during repletion periods when a basal sodium- and potassium-free ration was fed, with separate groups receiving additional sodium or potassium, or both.

The influence of the absence of sodium in the diet was first studied using potassium substituted for the sodium usually given in a salt mixture on an equimolar basis. Over a twenty-day period, the rats gained weight on this high potassium, sodium-free regimen. The experiment was then extended using rats divided into groups according to the presence or absence of sodium or potassium or both in their rations. All animals gained well over the twenty-day period except those on diets containing neither sodium nor potassium. The hearts of these animals were examined for the myocardial necrosis lesions reported by other authors studying potassium-deficient animals, but only one of the animals showed such lesions and they were minimal in extent.

In the second part of the experiment, the

adverse influence of sodium supplementation with potassium deficiency was studied. Rats prepared by the above-described protein depletion were given a basal ration without sodium or potassium for seventeen days. They all lost weight. They were all fed the basal ration plus calcium and magnesium salts in an experiment planned to last thirty days. One group (group 3) was given potassium alone. All rats of the first group (basal ration) survived but lost weight; 3 of the 5 animals in the second group (basal ration plus sodium) died before the end of the test period; and all animals of group 3 survived and gained weight. Examination of the animals' hearts showed only minimal lesions in some of group 1, and no lesions in 2 specimens from group 3. All hearts from the animals of group 2 showed severe necrosis.

In a third experiment, the animals were prepared in general as the others were previously and divided into four groups. Animals in group 1 were given no sodium or potassium. Group 2 animals were given the basal ration with potassium added and received daily subcutaneous injections of desoxycorticosterone. The animals in group 3 were given added sodium chloride only and those of group 4 were given the same sodium ration with daily desoxycorticosterone injections. The animals of group 1 again survived but lost weight. Those of group 2 gained weight and were healthy while those of the last two groups all died before the fourteenth day. At autopsy, the hearts and livers of the latter animals showed severe necrosis while those of the members of groups 1 and 2 were normal.

Experiment 4 was designed to produce more definite evidence of the "toxic" effect of increased sodium intake in potassium-deficient states. Animals that had the usual partial depletion of protein and potassium were again divided into groups. Group 1 rats were given the basal ration plus added sodium and potassium. An effort was made to develop heart lesions in the rats of group 2 by feeding a diet without potassium but with sodium over a fifteen-day period. From the fifteenth to nineteenth days these animals were able to withstand 5 cc. injections of isotonic saline or 5 per cent glucose. They were then given, on the twentieth day, 5 cc. injections of 4.66 per cent sodium chloride subcutaneously with the result that all died within twenty-four hours. The third and fourth groups received rations similar to the second group and were similarly treated with hypertonic saline on the twelfth day. The fourth group was given desoxycorticosterone in addition. All rats in these two latter groups died within forty-eight hours after the saline injections. Hypertonic saline injections were without effect, however, in protein-depleted rats that had not been specifically potassium depleted.

In an effort to establish the time necessary for these effects, two groups of rats were given potassium-free rations with equimolar amounts of sodium substituted for potassium salts. One group then received daily injections of hypertonic potassium chloride while the other received similar sodium chloride injections. The rats of the first group gained weight over the fourteen-day test period while those animals given sodium chloride died suddenly on the sixth to the eleventh day. At autopsy, the hearts of these latter animals showed widespread coagulation necrosis.

The results of these experiments furnish evidence that myocardial lesions will develop in the presence of potassium depletion. These, however, may be fairly insignificant if sodium is also depleted, but in the presence of added sodium in potassium-deficient animals, such lesions become more severe and, at least under the conditions of these experiments, may become lethal. French (*loc. cit.*) has also noted that the severity of myocardial fiber necrosis in potassium-deficient animals is influenced by acid-base balance and adrenocortical hormones as well as by sodium intake.

The authors noted that the cardiac lesions of potassium deficiency alone were loss of muscle fibers with cellular replacement while those in animals given a low potassium, high sodium diet showed hyaline or coagulative necrosis without cellular infiltration. The lesions, however, were located in the same subendocardial regions in both groups of animals. The authors suggest that the difference may be simply in the greater acuteness of the lesions in animals given a high sodium intake.

Since it had been observed (P. D. Boyer, H. A. Lardy, and P. H. Phillips, *J. Biol. Chem.* **149,** *529 (1943)*) that some enzymic reactions potentiated by potassium are inhibited by sodium, the authors speculate that some similar mechanism may account for their observations in these studies that sodium is a "toxic" ion in animals with potassium deficiency.

This investigation may provide an explanation for the frequent lack of correlation among clinical state, electrocardiographic changes, and serum potassium levels in man. It should also serve to emphasize the possible dangers of the use of sodium in the patient with potassium deficiency.

SOME STUDIES ON MINERAL NUTRITION

Several recent publications have added significantly to our knowledge of the requirements for and the metabolism of certain mineral components of foods. C. H. Burns, W. W. Cravens, and P. H. Phillips (*J. Nutrition* **50,** *317 (1953)*) have reported on requirements and interrelationships of sodium and potassium. Using chicks as their experimental animals, they fed semi-synthetic diets in which the levels of sodium and potassium could be varied at will. In their first experiment sodium was incorporated in the diet at levels of 0.02, 0.04, 0.10, 0.14 and 1.0 per cent of the ration. At all these sodium levels growth was poor and mortality high when the ration contained only 0.11 per cent potassium. Levels of 0.15, 0.23 and 0.77 per cent potassium supported better growth and tended to prevent mortality. At each of these higher potassium levels best growth was obtained when the diets contained between 0.10 and 0.14 per cent sodium. Growth depression and mortality were observed with the 0.77 per cent potassium diet containing 0.02 per cent sodium. Thus both sodium and potassium appeared toxic if the other element was present only in small concentrations.

A second experiment confirmed this conclusion. Sodium was incorporated in the diet at a level of 1.5 per cent and potassium at levels ranging from 0.15 to 1.5 per cent. With 0.15 per cent potassium mortality was 75 per cent, dropping to 50 per cent with 0.33 per cent potassium. The highest level of potassium (1.5 per cent) gave a mortality of only 8 per cent. Growth rates over a twelve-day period improved strikingly with the increase in potassium concentration.

A third experiment revealed that with diets containing 0.18 per cent or more sodium maximal weight gains were attained with 0.33 per cent potassium. On the other hand, a 0.10 per cent sodium level required 1.0 per cent potassium to support maximal growth. This would seem to demonstrate a slight sparing action of one element on the other. Further investigation showed this sparing effect to hold true only when either element was present in barely limiting amounts. Alterations in dietary sodium and potassium produced profound effects on the blood levels of the two elements. Blood sodium was raised by increasing the dietary sodium level; as blood sodium increased, blood potassium decreased. An increase in the level of dietary potassium usually increased the blood sodium level. The

requirements for sodium appeared to be a function of growth rate.

M. B. Patton and co-workers (*J. Nutrition* **50,** *373* (*1953*)) have added further data to the already impressive list dealing with human calcium and phosphorus requirements. They concluded that the calcium and phosphorus balances are more closely related to the absolute intakes of the two elements than to the calcium:phosphorus ratio of the ration.

Magnesium deficiency in the duckling has been studied by R. Van Reen and P. B. Pearson (*J. Nutrition* **51,** *191* (*1953*)). A basal diet of the semisynthetic variety, containing less than 1 mg. of magnesium per 100 g. of ration, was supplemented with magnesium at levels ranging from 0 to 120 mg. per 100 g. of diet. Low levels of the element gave rise to deficiency symptoms in an extraordinarily short time. The convulsive attacks observed differed from those in rats and mice in that they could not be precipitated by auditory stimuli. The magnesium required to sustain maximal growth was approximately 50 mg. per 100 g. of diet, compared with a chick requirement of about 40 mg. Considerable alterations in enzyme concentrations were observed in deficient animals. Alkaline phosphatase of brain tissue was reduced to 70 per cent of that found in control animals. Some reduction in DPNase also occurred. Cytochrome oxidase and inorganic pyrophosphatase remained unchanged in brain tissue. The liver content of niacin, riboflavin and pantothenic acid per 100 g. of fresh tissue was roughly the same in deficient and in control animals.

L. R. Arrington and G. K. Davis (*J. Nutrition* **51,** *295* (*1953*)) added 0.1 per cent molybdenum to a commercial ration and observed toxic effects when fed to rabbits. The relatively nonspecific symptoms of toxicity (anorexia, weight loss, alopecia, dermatosis, anemia and death) were similar to those observed in rabbits fed a ration low in copper. The addition of more copper to the molybdenum-containing ration prevented the onset of toxicity symptoms or effected a cure in animals showing the effects of excess molybdenum.

The rate of removal of fluorine from the rat femur has been investigated by R. F. Miller and P. H. Phillips (*J. Nutrition* **51,** *273* (*1953*)). During a six-week period of feeding a high dietary level of fluoride considerable amounts of this element were deposited in the skeleton. When this regimen was followed by one supplying very little fluoride, 33 per cent of the total bone fluorine was lost in a four-week period. After eight weeks on the fluoride-low ration 44 per cent of the element had been lost from bone. During an additional twenty-four-week period no further decline was observed. The authors conclude that bone contains two types of fluorine deposits, one which is rapidly removed during reduced intake, the other which has an extremely low turnover rate.

METABOLIC INFLUENCES ON TOOTH DEVELOPMENT DURING PREGNANCY

Numerous investigators have studied prenatal metabolic influences which affected the dental tissues. M. Diamond and J. P. Weinmann ("The Enamel of Human Teeth," *Columbia University Press, New York* (*1940*)) concluded that the metabolic disturbances responsible for enamel hypoplasia in man occurred primarily during the last half of uterine life and before the end of the first year of life. H. Mellanby (*J. Dent. Res.* **20,** *489* (*1941*)) noted abnormally shaped incisors and molars, and retarded tooth development in the offspring of rats made deficient in vitamin A during the gestation period. M. W. Evans (*Med. J. Australia* **2,** *225* (*1944*)) reported dental defects in a

high percentage of children born of mothers who had German measles during pregnancy.

The effects of certain prenatal systemic disturbances, including diabetes, on the development of the rat fetus, especially as related to tooth development, have been studied by S. J. Kreshover, O. W. Clough, and D. M. Bear (*J. Dent. Res.* **32,** *246 (1953)*). Even with our modern methods for the treatment of diabetic subjects, their stillbirth rate is about twice that of non-diabetic pregnancies. Although various abnormalities have been reported in the young of both controlled and uncontrolled diabetic patients, seemingly little attention has been given to its possible influence on tooth development. Since alloxan is capable of causing necrosis of the pancreatic islets of Langerhans, it can be used effectively to produce an experimental type of diabetes at specific dates after the beginning of pregnancy in experimental animals.

To study alloxan-produced diabetes in rats, 44 normal female rats, on a normal diet, were segregated after mating. Five were used as controls and 39 were injected intraperitoneally with a freshly prepared 10 per cent solution of alloxan at an average dosage of 155 mg. per kilogram of body weight. To provide a reasonably exact and complete record of the "diabetic" status, blood analyses and repeated urinalyses were made. The experimental animals were grouped according to the first, second and third weeks of pregnancy; injections of protamine zinc insulin were administered as required to maintain the rats in moderate degrees of hyperglycemia and glycosuria. The newborn of each successful pregnancy were weighed and then killed at intervals of one, seven, twenty-one, forty and sixty days. The jaws and pancreas of the offspring of experimental animals and controls were removed for gross and histologic study. Following pregnancy, the females were killed and the teeth and pancreas were examined.

The distribution of "unsuccessful pregnancies" in relation to the time of alloxan administration warrants attention. Seemingly it is possible that early injection could interfere with conception or cause fetal resorption. There was found to be a correlation between the time of alloxan injection and the percentage of delivered living and dead young. In the group receiving alloxan during the first, second and third weeks after mating, there was a total of 6 dead and 13 living offspring in two litters, 4 dead and 49 living in 9 litters, and 3 dead and 112 living in 11 litters, respectively. Among the control rats, all 5 females delivered a total of 53 living and no dead offspring. From the findings presented in this report, it would appear that the alloxan not only influenced the course of pregnancy, but that the earlier in pregnancy it was given, the more serious were its effects.

The living offspring of the alloxan-injected rats were comparable in weight and blood sugar level to those of the control group. No gross dental changes were manifest in any of the experimental animals, but histologic examinations showed a significantly large number with abnormalities of enamel formation. Most abnormalities were seen in the incisor teeth although similar changes were noted frequently in molar teeth undergoing development. With few exceptions, the teeth of the alloxan-injected rats showed marked changes in the ameloblasts, organic matrix of the enamel, odontoblasts and pulp. A common finding was the presence of enamel-like deposits on the surface of the organic enamel matrix. In addition, most of the alloxan-injected rats showed pulpal hyperemia or hemorrhage. Whereas the changes in the enamel organ were confined to the incisor teeth the pulpal lesions were found also in the molars. No consistent periodontal abnormalities were noted except for occasional hyperemia or hemorrhage. Whether this high percentage of developmental dental abnormalities in pregnant rats injected with alloxan and their offspring is caused by an uncompli-

cated disturbed hormone function of the pancreas, by alloxan toxicity per se, or by some unknown factor is conjectural. Despite the evidence offered by C. E. Friedgood and A. A. Miller (*Proc. Soc. Exp. Biol. Med.* **59,** *61* (*1945*)) that appreciable amounts of alloxan may cross the placenta from mother to fetus, and that fetal hyperglycemia paralleling that of the mother is present up to the time of parturition, the present study gave no histologic indication of pancreatic damage in the young.

Further studies of the influence of prenatal disturbances are reported by S. J. Kreshover and O. W. Clough (*J. Dent. Res.* **32,** *565* (*1953*)). Twenty-eight pregnant rats, grouped according to stages of pregnancy, were subjected to artificially induced fever by means of an incubator equipped with ventilation and humidity. The animals were kept in the incubator for periods up to forty-eight hours and their rectal temperatures were maintained within the range of 102 to 105° F. Four non-pregnant animals received the same treatment. The teeth of these pregnant rats showed changes ranging from ameloblastic dysfunction to complete cellular degeneration with the arrest of the formation of the organic matrix of the enamel. No significant changes were noted in the dentin. The offspring of these mothers which were subjected to the elevated temperatures during pregnancy had similar dental abnormalities. Additional disturbances were observed in the dentin of the teeth of the offspring. Maternal deaths were higher when the period of fever was imposed late in pregnancy than when a similar period was imposed in the early days of pregnancy. It is interesting to note that the abnormalities observed in the heat-treated females and their offspring were almost identical to those observed in the offspring of alloxan-treated rats.

It would seem from these studies that the elevated fetal temperature which can be assumed to have occurred simultaneously with that of the maternal organism may affect the tooth development in the offspring in a manner similar to that which occurred in the mother. Though no information is available regarding the temperature of the young *in utero*, it seems reasonable to suppose that the degree of temperature is comparable to that of the mother.

The current series of investigations have sought to explore the existence of etiologic factors related to tooth development which are not considered to be limited by the selective ability of the placental barrier. The present studies suggest that, even in the case of bacterial infection in the mother, there is a definite possibility that the injurious effects on the teeth of the offspring may be mediated by some endocrine or general metabolic pathway rather than by direct placental transfer of infection. The responses noted to the febrile conditions which were artificially produced are particularly interesting by reason of the frequent febrile reactions which are observed in a wide variety of infectious disturbances. The abnormalities described in these experiments were relatively gross ones at the histologic level, although it was noted that they were insufficiently severe to produce clinically demonstrable lesions. There is a definite possibility that the experimental periods were not long enough to permit the eruption of the areas which were disturbed during development.

It is interesting to speculate on the possible relation of such developmental abnormalities on the future history of these teeth when subjected to the oral environment for prolonged periods. There would seem to be ample justification for further studies of this kind where the observational periods are extended to determine the effect of such disturbances on the caries-susceptibility of the teeth. Likewise, there is a real need for this type of experiment to be extended to determine the effect of such abnormalities on the composition of the

inorganic components of the enamel and dentin. Nor should the effect of the deficiency of the various nutrients during tooth development be overlooked. Somewhere in the interrelationships between metabolic processes and tooth development is hidden the reason why some teeth are highly resistant and others are highly susceptible to tooth decay when exposed to the same oral environment. At present it is impossible to evaluate any one of these interrelationships, which fact should stimulate the collection of a substantial body of experimental data in this area.

ASCORBIC ACID AND ADRENOCORTICAL FUNCTION

The high concentration of ascorbic acid within the adrenal cortex and the rapid decline of adrenal ascorbic acid following the administration of ACTH has suggested an important role for this vitamin in hormone production (*Nutrition Reviews* **3,** *37* (*1945*)). Attempts to show a failure of production of adrenal cortical steroids in scurvy by determinations of urinary 17-ketosteroids and corticoids have not been successful. Studies in monkeys and guinea pigs (*Ibid.* **11,** *150* (*1953*)) have suggested that there may be a small decline in steroid excretion during the asymptomatic period on a scorbutogenic diet. However, with the onset of symptoms a greater than normal excretion occurs. Moreover, the response to ACTH is not abnormal in ascorbic acid-deficient animals. The response of scorbutic patients to ACTH is normal as judged by 17-ketosteroid excretion and eosinophilopenic response (H. S. Treager, G. J. Gabuzda, N. Zamcheck, and C. S. Davidson, *Proc. Soc. Exp. Biol. Med.* **75,** *517* (*1950*)).

The work of B. E. Clayton and F. T. G. Prunty (*J. Endocrinol.* **9,** *370* (*1953*)) provides further evidence that adrenal cortical hormone production is not decreased in scurvy. Guinea pigs were placed on a scorbutogenic diet for nineteen or twenty days, by which time measurements of adrenal ascorbic acid indicated very low levels. ACTH in a dose of 20 mg. per day divided into eight injections was administered subcutaneously for four days. The excretion of 17-ketosteroids by the ACTH-treated and control guinea pigs (the latter receiving saline solution) was measured before, during and after the treatment period. An increase of 17-ketosteroids of from 40 to 206 per cent occurred during ACTH treatment. A less definite increase in 17-ketosteroids which occurred in the control group probably could be accounted for on the basis of scurvy alone. The brief ACTH treatment had no effect on survival.

The effect of adrenalectomy on survival in scurvy was also investigated. Adrenalectomized guinea pigs deprived of ascorbic acid with one exception died within one week. The 17-ketosteroids in such animals failed to have a preterminal peak of excretion. Despite the early death of scorbutic-adrenalectomized guinea pigs there was no acceleration in the bony lesions of scurvy, and hemorrhages were few.

Experimental studies which have used the 17-ketosteroids as an index of adrenal activity are subject to certain criticisms. It is known that the testis as well as the adrenal cortex contributes to the excretion of 17-ketosteroids. Unless female animals are used exclusively some uncertainty exists concerning the origin of the measured steroid. The physiologic significance of the 17-ketosteroids is still obscure. It would seem that they represent both impotent by-products of corticoid synthesis and also the end stage of one path of corticoid destruction. For this reason it is difficult to correlate the level of active hormone production directly with 17-ketosteroid excretion. The methods of measuring

urinary corticoid excretion (formaldehydogenic and reducing steroids) are only slightly more specific. An additional uncertainty is introduced when measurements are performed on sick animals. When renal function is impaired, evidence suggests that steroid clearance may fall greatly. These comments are not meant to minimize the contributions of earlier workers, but to indicate the need for confirmation of continued corticoid production in scurvy with methods not subject to these potential pitfalls.

The measurement of 17-hydroxycorticosteroids in carefully purified extracts of blood by means of the Porter-Silber reaction is the best means available of assessing adrenal cortical activity. A. K. Done, R. S. Ely, L. R. Heiselt, and V. C. Kelley (*Proc. Soc. Exp. Biol. Med.* **83,** *722 (1953)*) have applied this method to scorbutic guinea pigs. The level of 17-hydroxycorticosteroids in 17 normal guinea pigs was found to be 32.9 ± 4.6 micrograms per cent. This value is substantially higher than the level in normal human beings. Blood was obtained from 18 guinea pigs after they had ingested an ascorbic acid-deficient diet for sixteen to twenty-five days. The plasma 17-hydroxycorticosteroids of these animals averaged 301.9 ± 0.43 micrograms per cent which was almost ten times the control value. When 5 guinea pigs fed the scorbutogenic diet for twenty days were given 25 mg. per day of ascorbic acid for five days the mean plasma 17-hydroxycorticosteroids fell to 68.7 ± 19.4 micrograms per cent. These studies, therefore, confirm the *increased* level of adrenal hormones in the blood predicted on the basis of the study of urinary metabolites. The ten-fold increase in plasma levels over normal is actually greater than that observed in the urinary steroids. Done and his collaborators have been cautious in the interpretation of their data. They point out that an increased level of plasma steroid might indicate interference with utilization, conjugation, or excretion of 17-hydroxycorticosteroids rather than increased production.

The influence of ascorbic acid deficiency in the metabolism of cortisone has been studied by H. Bacchus (*Endocrinology* **53,** *441 (1953)*). This investigator has incubated cortisone *in vitro* with pieces of liver (size not specified) in phosphate buffer. After three hours the amount of Porter-Silber reactive material was measured. In this test system liver from guinea pigs on an ascorbic acid-deficient diet for nineteen days destroyed 53 per cent of 17-hydroxycorticosteroid material. Animals on the deficient diet for only five days destroyed 54 per cent. The *in vitro* addition of ascorbic acid did not lower cortisone destruction. The author interprets these observations as support for his theory that ascorbic acid actually protects cortisone against breakdown. Certainly these observations do not suggest impaired ability to utilize corticosteroids in scurvy.

In summary, the evidence would appear conclusive that the adrenal retains the ability to produce normal and even increased amounts of corticosteroids in symptomatic scurvy. The possibility that the small amount of ascorbic acid remaining in the adrenal of the scorbutic animal is necessary for steroidogenesis cannot be answered at the present time.

THE METABOLISM OF RADIOACTIVE NIACIN AND NIACINAMIDE IN THE RAT

The pathways of excretion of niacin and niacinamide have been studied extensively. W. A. Perlzweig, F. Rosen, and P. B. Pearson (*J. Nutrition* **40,** *453 (1950)*) measured the excretory products of these substances in a variety of species. In these

studies and in most of the others reported in the literature the experimental approach has consisted of administering relatively large quantities of the vitamins and then measuring the excretion of various metabolites in the urine. The possibility exists that under these conditions one is actually dealing with a detoxication mechanism and not necessarily the normal metabolic disposal of physiologic quantities of niacin or niacinamide. Also, the existing analytic methods limit the metabolites which may be determined.

These objections have been overcome in part by the experimental approach used by B. C. Johnson and P.-H. Lin (*J. Am. Chem. Soc.* **75,** *2971, 2974 (1953)*). These workers utilized paper chromatography technics and C^{14}-labeled niacin and niacinamide. With these procedures it was possible to inject physiologic quantities of niacin and niacinamide into rats and to measure a large number of metabolites in the urine.

The first paper by these workers describes the chromatography technics. They used paper strips in a solvent system which consisted of the upper phase of a mixture of 45 per cent n-butanol, 5 per cent acetone, and 50 per cent water. After the strips were developed and dried, a variety of detection methods were applied. The cyanogen bromide reaction was utilized to locate niacin, niacinamide, and nicotinuric acid. Coenzymes I and II, niacinamide ribosephosphate, niacinamide riboside, N^1-methylnicotinuric acid betaine, and N^1-methylniacinamide chloride were located by fluorometric technics. The position of N^1-methyl-2-pyridone-5-carboxylamide was detected by elution of successive 3 cm. sections of the paper strip followed by measurement of the absorption of the eluate at 260 millimicrons.

Bioautographic methods were employed for niacin, niacinamide, nicotinuric acid, trigonelline, and niacinamide riboside and mononucleotide. This method utilized growth of the yeast *Torula cremoris*. The basal medium which did not contain any of the niacin compounds was inoculated with the organism and allowed to solidify. The strips were then placed on the surface of the medium for ten minutes and removed, after which the mediums were incubated for twenty-four hours. Growth zones were observed at spots which corresponded to the positions of the active substances on the paper strip. This method was more sensitive than any of the chemical methods employed.

In order to detect radioactive material on the paper strip, radiographs were prepared. The dry strips were placed between two sheets of x-ray film. The exposure time varied with the activity of the material. The radioactive materials would darken the x-ray film and the R_f values could be determined. The degree of darkening also served as a rough quantitative estimate of the amount of radioactivity. A number of experiments were then conducted in which pure compounds were chromatographed and the R_f values were determined.

In the next experiments Lin and Johnson injected C^{14} carboxyl-labeled niacin and niacinamide into rats. The weight of the rats varied from 195 to 325 g. Two dosage levels of niacin and niacinamide were used. Some animals were given a high level of from 1.5 to 2.5 mg. per rat. Other rats received a more physiologic level of 230.6 micrograms of niacin and 236.7 micrograms of niacinamide per rat per day for a four-day urinary collection period. The latter animals received a purified diet free of niacin. The animals receiving the high dosage level were fasted for the two-day collection period. There were 3 rats receiving the large dose of niacin and 3 receiving the large dose of niacinamide. Only 1 rat was given the smaller dose of niacinamide and one the smaller dose of niacin.

The urine collected from these animals was subjected to paper chromatography

and radioautographs were prepared. In some experiments the active spots indicated by the radioautograph were cut from the strip and counted on a Q gas counter and scaler.

With the high dosage level of niacin or niacinamide a large number of spots of radioactivity were observed on the radioautographs. There was one unidentified spot on the radiograph of urine from the rat given niacin and 3 for the rat given niacinamide. These accounted for 7.74 per cent and a total of 12.61 per cent of the total urinary activity respectively. The number of counts, as per cent of the total urinary counts, which were from identified metabolites of administered niacin were: coenzyme I, 3.50; N^1-methylniacinamide, 2.73; nicotinuric acid, 60.93; niacin, 24.36; N^1-methyl-2-pyridone-5-carboxylamide, 0.48; and niacinamide, 0.26. Corresponding data for the rat receiving niacinamide were: coenzyme I, 6.08; N^1-methylniacinamide, 66.94; nicotinuric acid, 0.90; niacin, 0.59; N^1 - methyl- 2 -pyridone- 5 -carboxylamide, 2.43; and niacinamide, 4.10. Thus the large dose of niacin was excreted chiefly as nicotinuric acid and unchanged niacin, whereas the large dose of niacinamide was excreted chiefly as N^1-methylniacinamide. The data indicate little interconversion of niacin and niacinamide under the conditions of high dosage.

When the more physiologic quantities of niacin and niacinamide were administered the results were considerably different. Fewer spots appeared on the radioautographs. Under these conditions N^1-methylniacinamide appeared to be the chief excretory product of both niacin and niacinamide. There was some nicotinuric acid excretion by the rat given niacin and both rats appeared to excrete some labeled nucleotides.

These results indicate that nicotinuric acid may be considered a detoxication product of niacin in the rat rather than a normal metabolic product. When given in physiologic quantities the major excretory product of both forms of the vitamin appears to be N^1-methylniacinamide. These results emphasize the fact that when large doses of a vitamin are given the observed pattern of excretory products may not represent the normal metabolic disposal of the vitamin.

COMPARATIVE URINARY EXCRETION OF D- AND L-ISOMERS OF AMINO ACIDS

It has been long recognized that D- and L-isomers of the amino acids follow different metabolic pathways within the body and that L-forms are more completely and efficiently metabolized. Studies of the rates of intestinal absorption from isolated loops of intestine of rats have demonstrated that the L-isomers of all amino acids investigated by this means are absorbed at a rate exceeding that of the D-forms (Q. H. Gibson and G. Wiseman, *Biochem. J.* **48,** *426 (1951)*; *Nutrition Reviews* **9,** *343 (1951)*). These observations indicate that there exist specific mechanisms of active absorption from the intestine of L-amino acids, while D-forms probably pass through the intestinal mucosa by simple processes of diffusion.

A recent investigation of R. F. Crampton and D. H. Smyth (*J. Physiol.* **122,** *1 (1953)*) has been concerned with the urinary excretion of stereoisomers of the amino acids and has sought evidence of stereochemically specific mechanisms for the absorption of amino acids by the renal tubules. These experiments were done in cats and the urinary clearances of creatinine and amino acids were determined. Racemic mixtures of amino acids were injected intravenously under standard conditions.

Blood samples were obtained through a cannula placed in the common carotid artery. Urine was collected through a urethral cannula designed to prevent accumulation of residual urine in the bladder. The animals were allowed water but no food for periods of twelve to eighteen hours before the initiation of experiments. Barbiturate anesthesia was utilized.

The amino acids studied included alanine, methionine and histidine. The quantity of L-isomers of alanine and methionine in specimens of blood and urine was determined manometrically, utilizing a dried preparation of L-amino acid oxidase prepared from *Neurospora crassa*. Similarly, the quantitative measure of D-isomer was achieved by use of D-amino acid oxidase obtained from pig kidney. In experiments with histidine, the quantity of the L-form in urine was determined through the action of histidine decarboxylase and the D-form was estimated by the difference between total histidine determined colorimetrically and L-histidine assayed by the enzymatic procedure.

With each of the amino acids injected it was determined that the quantity of D-isomer excreted in the urine exceeded that of the L-form, while in blood plasma there were uniformly greater concentrations of the L-isomers. It appeared that the lower values for D-isomers in plasma after injection of racemic mixtures was probably due to the more rapid rate of urinary excretion of this stereoisomer. The greater plasma concentration of L-isomer would indicate similar greater concentration in glomerular filtrates, implying that there must be preferential reabsorption of this isomer in renal tubules.

Determination of the renal clearance of the L-isomers of alanine and methionine provided confirmatory results. When the concentration in plasma was relatively low, the quantity appearing in the urine was little different from that occurring when no amino acid was injected. When the plasma concentration was elevated above a certain critical level, there was a rapid increase in the quantity escaping into the urine. The plasma threshold, thus determined, for L-alanine was 0.4 mg. per milliliter and for L-methionine was 1.3 mg. per milliliter. Only a process of active tubular reabsorption of the stereoisomers could account for these findings.

In contrast, the D-isomers of these amino acids demonstrated very different types of renal clearance. Even at lowest plasma levels 60 per cent of the D-alanine in glomerular filtrates appeared in the urine. Although less of the filtered D-methionine escaped into the urine at low plasma levels than was the case with D-alanine, the concentration of D-methionine in urine always exceeded that in plasma. These findings seemed to indicate that some of the D-isomers did again return to the blood stream in the renal tubules. However, the quantities were relatively small and could conceivably be accounted for by processes of simple diffusion.

The rates of diffusion of the stereoisomers of alanine through membranes were determined. It was found that D- and L-alanine passed through an artificial membrane, cellophane, at identical rates. Since it was impossible to prepare a biologic membrane from renal tubular epithelium, the rates of diffusion were compared in rat intestine suspended in a buffer at reduced temperature (10°C.). Under these conditions it was found again that the diffusion rates for D- and L-alanine were of the same magnitude. The authors concluded, therefore, that since rates of diffusion of the stereoisomers were similar across these barriers, there must exist specific active mechanisms for reabsorption of L-isomers of amino acids in the renal tubules.

These observations coupled with those of active intestinal absorption of L-amino acids

provide important evidence of the greater availability and nutritional value of L-amino acids as compared with the D-isomers. Although other evidence indicates that D-amino acids may be readily metabolized by various tissues or converted to L-forms, the inability of the intestine to absorb actively D-amino acids and of the kidney to retain them affords substantial explanation for their relatively poor biologic value.

GENETIC AND ENVIRONMENTAL FACTORS IN GROWTH

One of the primary concerns of the nutritionist is the phenomenon of growth. He makes use of growth as one index of dietary adequacy, whether he is comparing protein quality, measuring the requirement of an animal for a particular vitamin, studying the effects of antimetabolites, or pursuing any of the many other problems which engage the nutritionist. The role of nutrients in the growth of an animal is implicit even in studies dealing with the function of such nutrients in intermediary metabolism. One purpose of such investigations is the elucidation of the mechanism by which a given nutrient exerts its growth-promoting action.

The growth of any organism, be it protozoon or mammal, is determined first of all by its genetic makeup. At the very least it is true that genetic constitution determines the upper limit of growth; it determines the maximum growth which may be obtained under ideal conditions. The mechanisms which operate to establish hereditary growth patterns are still little understood. It is known (largely from the work on *Neurospora*) that genes are concerned in the establishment and the maintenance of enzyme systems. It seems entirely plausible that the qualitative and quantitative aspects of an organism's enzymatic pattern may play an important role in determining growth limitations. Another possibility is that inherited endocrine patterns may determine in part the hereditary growth limitations. This could, of course, be effected by genetic control over those enzyme systems concerned with the synthesis of humoral substances.

Living organisms are capable of realizing their full growth potentialities only under suitable environmental conditions, chief among which are the quantity and quality of nutrients available. It is immediately obvious to any nutritionist that no organism can realize its maximum growth potentialities unless supplied with adequate quantities of calories, amino acids, vitamins and minerals. Conversely, providing an animal with an ideal food supply and housing it under ideal conditions should result in the attainment of maximal growth rates as determined by hereditary factors.

In this context, two lectures given before the Harvey Society are of special interest. One of these, entitled "Growth and Adrenocorticotropic Hormones of Anterior Pituitary" by C. H. Li (*Harvey Lectures* **46,** *181 (1950/51)*), deals with the action of two pituitary hormones on growth and other metabolic processes. In this lecture, Li has summarized some of his work and the work of others on the chemical and physiologic properties of the pituitary hormones. The growth hormone was isolated in pure form by Li and his associates (Li, H. M. Evans, and M. E. Simpson, *J. Biol. Chem.* **159,** *353 (1945)*), while A. E. Wilhelmi, J. B. Fishman, and J. A. Russell (*Ibid.* **176,** *735 (1948)*) described a simpler method for isolating the hormone in relatively large yields and in crystalline form. It has a molecular weight of 44,000 and an isoelectric point of pH 6.85. As is characteristic of physiologically active proteins, the hormone is heat-labile and sensitive to the action of digestive enzymes. In the rat hypophysectomy leads to cessation of

growth, although very young rats may grow slowly for some days following the operation. There is a marked thinning of the epiphyseal cartilage plate, indicative of retardation or cessation of chondrogenesis and osteogenesis. Administration of pure growth hormone induces, even in animals lacking adrenals, thyroids, or gonads, renewed growth and resumption of chondrogenesis and osteogenesis. Simultaneous administration of thyroid hormone and growth hormone leads to a synergistic effect upon these processes.

Some indication of how the growth hormone exerts the physiologic activities after which it is named comes from the observation that it leads to retention of nitrogen and also to a reduction of circulating amino acids in blood plasma. Simultaneously, extra protein is deposited along with water. Concurrent with the deposition of protein and water there is a diminution of carcass fat content. There is now ample evidence from several species of experimental animals that the growth hormone is diabetogenic. Administration of growth hormone may induce glycosuria, hyperglycemia and under some conditions permanent diabetes. *In vitro* it inhibits the synthesis of glycogen by isolated diaphragm. In this respect it opposes the action of insulin. The administration of growth hormone has been shown to increase the blood and urinary concentrations of ketone bodies during fasting. It also increases phospholipid turnover in the liver and leads to an increase in liver fat concentration in fasting animals. Furthermore, it decreases the amount of carcass fat. Growth hormone therefore effects a shift in the animal's metabolic pattern, increasing protein synthesis (or retarding its breakdown). It accelerates fat metabolism while inhibiting the oxidation of glucose and its transformation into muscle glycogen. Although such experiments must necessarily be carried out with hormone doses larger than those normally secreted, they nevertheless suggest the effects of the hormone in the normal, intact animal. If genetic factors determine the rate of endocrine output, then it follows that they also regulate growth and other metabolic processes.

While the growth hormone of the pituitary enhances growth, the adrenocorticotropic hormone is known to retard growth, chondrogenesis and osteogenesis. At the same time, administration of ACTH increases the amino acid content of blood plasma and enhances urinary nitrogen excretion. These observations can be explained largely in terms of the increase in gluconeogenesis induced by the adrenocortical hormones. In some respects the growth hormone and ACTH have similar functions. Both oppose the insulin effect on glycogen synthesis in isolated diaphragm. Both increase liver fat and phospholipid turnover.

Another lecture in the Harvey series, entitled "The Interplay of Heredity and Environment in the Synthesis of Respiratory Enzymes in Yeast," by B. Ephrussi (*Harvey Lectures* **46,** *45 (1950/51)*) deals more directly with the distinction between genotype and phenotype. Ephrussi showed that baker's yeast plated on agar gives rise not only to the expected yeast colonies but also produces in addition about 1 per cent of much smaller colonies consisting of smaller than normal cells. These small cells appear to be a stable mutant, since on replating they give rise only to small cells.

A series of interesting experiments on the fundamental metabolic differences between the normal and small strains of yeast are recounted in this lecture. If supplied with a limiting amount of glucose, the normal cells grow faster than the small ones in the presence of oxygen. Under anaerobic conditions the normal cells and the small cells show the same rate of growth. In the presence of oxygen the normal cells cause 80 to 90 per cent of the glucose to disappear by respiration and only 10 to 20 per cent by fermentation. In the absence of oxygen,

fermentation is greatly increased. On the other hand, the small cells ferment about 98 per cent of the glucose regardless of the presence or absence of oxygen. The normal cells are inhibited by cyanide, while the small cells appear not to be affected. These experiments suggest a difference in the reliance of the two types of cells upon those metabolic pathways which involve cytochrome oxidase. Spectroscopic examination showed cytochrome c to be present in both types of cells, although aeration did not affect the cytochrome c band of the small cells as it did those of the normal cells. The characteristic absorptions of cytochromes a and b were lacking in the small cells. It has been suggested that one of the cytochromes a may be identical with cytochrome oxidase, while one of the cytochromes b may be identical with succinic dehydrogenase. The mutant cells apparently have not inherited those genes which control the synthesis of cytochrome oxidase and succinic dehydrogenase.

Micromanipulation of single cells showed that a mother cell may give rise both to normal and to small daughter cells. This observation, along with numerous crossing experiments, indicates that the synthesis of these respiratory enzymes in yeast is controlled both by genetic factors located in the nucleus and by particulate cytoplasmic components endowed with genetic continuity. Additional experiments showed that within the limits imposed by the nuclear and cytoplasmic genic material, environmental changes such as oxygen concentration may dramatically influence the enzymatic make-up of the yeast cell.

THE EFFECT OF CHOLINE ON FATTY ACID OXIDATION

The lipotropic effect of choline has been generally ascribed to a stimulation of the rate of transfer of fatty acids from the liver to tissue fat depots. The transport form of fatty acids has been assumed to be choline-containing phospholipids. A considerable body of data indicates that this is not the correct explanation. C. Entenman, I. L. Chaikoff, and D. B. Zilversmit (*J. Biol. Chem.* **166,** *15 (1946)*) studied the metabolism of plasma phospholipids in normal dogs and in dogs in which the liver had been removed from the circulation, but left *in situ*. Phospholipids containing radioactive phosphorus were injected into these animals and the rate of disappearance was determined. Whereas in normal dogs plasma phospholipids were completely turned over in from six to ten hours, their turnover time was prolonged to from thirty-three to one hundred and sixty hours in the operated animals. Also, the concentration of plasma phospholipids as determined chemically did not appreciably decrease in these dogs.

Earlier experiments by M. C. Fishler, C. Entenman, M. L. Montgomery, and I. L. Chaikoff (*J. Biol. Chem.* **150,** *47 (1943)*) had shown that dogs deprived of their livers synthesized only negligible quantities of plasma phospholipids from injected radioactive inorganic phosphate. Thus it appears that the liver is the chief organ for both synthesis and degradation of plasma phospholipids. The earlier concept of the lipotropic effect of choline assumes that plasma phospholipids are broken down in the tissue depots and the liberated fatty acids are used for synthesis of neutral fat. The observation that only negligible quantities of plasma phospholipids are removed from the circulation in the dog without a functional liver is in direct opposition to this concept.

In other experiments D. B. Zilversmit, C. Entenman, and I. L. Chaikoff (*J. Biol. Chem.* **176,** *193 (1948)*) reported the effects of choline administration to dogs on the turnover time of liver and plasma phos-

pholipids as measured with radioactive phosphorus. The administration of choline increased the turnover of liver lecithin but did not affect the turnover of plasma lecithin or sphingomyelin. The authors concluded that choline does not act by increasing fat transport via plasma phospholipids but rather by stimulation of the utilization of fat within the liver itself.

This problem has been approached directly by C. Artom (*J. Biol. Chem.* **205,** *101 (1953)*) in a study of the effect of choline on the oxidation of fatty acids by the liver. The experimental approach consisted of incubating C^{14}-labeled stearic or palmitic acid with liver preparations from rats in various states of choline nutrition and measuring the amount of $C^{14}O_2$ produced. Male albino rats were given a basal diet of casein, 5 g.; dextrin, 42 g.; sucrose, 42 g.; Crisco (hydrogenated vegetable fat), 4 g.; cod liver oil, 1 g.; salt mix, 4 g.; Ruffex, 2 g.; thiamine, 0.4 mg.; riboflavin, 0.8 mg.; pyridoxine, 0.4 mg.; niacin, 5 mg.; calcium pantothenate, 2.5 mg.; para-aminobenzoic acid, 2.5 mg.; inositol, 10 mg.; folacin, 0.1 mg.; menadione, 0.5 mg.; biotin, 0.05 mg.; and vitamin B_{12}, 0.02 mg. This basal diet was supplemented with either 1 per cent guanidoacetic acid (choline-deficient diet) or 0.4 per cent choline chloride. In some experiments animals were given two intramuscular injections of choline chloride one and one-half and one-half hours before death. Other rats were fed a stock diet which contained 25 per cent crude mixed proteins and 5 per cent fat.

The rats were given these diets for varying periods of time, after which they were killed by decapitation and the livers were removed. Liver slices and homogenates were prepared. In some experiments the homogenates were centrifuged at 2000 g. and the sedimented particles were used in the incubation procedures. Also, in certain experiments mitochondrial preparations were made from the liver homogenates. These liver preparations were incubated for three hours with stearic acid-1-C^{14} or palmitic acid-1-C^{14}. After the incubation period, sulfuric acid was added from the sidearm of the Warburg-type flask and the liberated carbon dioxide was trapped by the sodium hydroxide on the filter paper in the center well. The carbon dioxide was precipitated as barium carbonate and counted with a Q gas flow counter. The results are reported as counts per minute per gram of tissue, per milligram of nitrogen or per whole liver of 100 g. of rat. In addition, phospholipids, neutral fat, and in some experiments choline-containing phospholipids, were determined chemically.

In the first experiments slices and homogenates of livers from rats fed the stock diet and from rats fed the choline-deficient diet were incubated with C^{14}-stearic acid. Homogenates from both types of animals were also incubated with C^{14}-palmitic acid. Slices and homogenates behaved quite similarly and the results of these experiments were clear-cut. Livers from choline-deficient rats produced approximately one-fifth as much $C^{14}O_2$ from either palmitic or stearic acid as did livers from normal rats when calculated on a per gram of tissue basis. Similar but less marked differences were obtained when the results were calculated per milligram of nitrogen or per whole liver. Results of chemical determination showed that livers from choline-deficient rats contained reduced quantities of phospholipids and greatly increased amounts of neutral fat as compared to normal rats.

The next experiments utilized liver slices and homogenates from rats fed the choline-deficient diet, the basal diet supplemented with choline, and each of these diets with choline injected one and one-half and one-half hours before the animal was killed. These liver preparations were incubated with 1-C^{14}-stearic acid. The results of these experiments clearly demonstrated that either feeding or injecting choline markedly stimulated the oxidation of stearic acid by

the liver preparations. The injection of 100 micromoles of choline was more effective than the feeding of 0.4 per cent choline. This stimulation of stearic acid oxidation by injected choline was observed in rats which had received the choline-deficient diet for one hundred and fifty days, at which time the liver was stated to be extensively fibrotic.

In other experiments it was found that the oxidation of palmitic acid by liver homogenates was stimulated by choline feeding or injections in a manner quite similar to the effect on stearic acid oxidation.

Experiments were next conducted in which the liver homogenates from rats fed the choline-deficient diet and similar animals injected with choline were further fractionated. Washed particles, obtained by centrifugation, and mitochondria were used. It was found that under these conditions the stimulation of fatty acid oxidation by choline injections was just as marked as when homogenates were used. This observation is important because it shows that the choline effect was not due to a change in the permeability of the cell membrane to the fatty acids.

In the final experiments the amount of C^{14}-acetoacetic acid produced from labeled palmitic and stearic acid was determined. Slices and homogenates were prepared from livers of choline-deficient rats, rats fed the stock diet, and rats fed the basal diet plus choline and injected with choline before death. The amount of C^{14} present in acetoacetate was considerably less than the amount present in carbon dioxide. Choline administration also increased the amount of labeled acetoacetate to about the same extent as the increase in $C^{14}O_2$ production.

The addition of choline chloride, phosphorylcholine, betaine aldehyde, or betaine *in vitro* to the incubation mixtures of fatty acids and liver did not increase $C^{14}O_2$ production regardless of the nutritional status of the animal.

If these results can be shown to be applicable to the intact animal they may allow an explanation for the lipotropic effect of choline. Choline may function by increasing the oxidation of fatty acids in the liver, thereby preventing the accumulation of fat.

Many additional experiments are indicated. The observation that choline added *in vitro* did not affect fatty acid oxidation suggests, as pointed out by the author, that not free choline but rather some choline derivative functions in fatty acid oxidation. Considerable circumstantial evidence suggests that this derivative is a phospholipid, but this remains to be proved. Also, the possible participation of choline oxidase in fatty acid oxidation is an intriguing speculation. It has been shown that the choline oxidase content is low in fatty livers (P. Handler and F. Bernheim, *J. Biol. Chem.* **144,** *401 (1942)*). If one considers that the removal of fat from the liver requires not only an adequate supply of choline but also an adequate turnover rate of some choline-containing compound it may allow a different approach to the biochemistry of lipotropic factors.

DIETARY PROTEIN AND REPRODUCTION IN RATS

There are few reports in the literature of the specific influence of dietary protein on the reproductive process in female rats, aside from the low-dietary protein studies of H. R. Guilbert and H. Goss (*J. Nutrition* **5,** *251 (1932)*) and the protein-free studies by W. H. Seegers (*Am. J. Physiol.* **119,** *474 (1937)*). These early studies generally indicated that a minimum dietary level of 6 per cent of casein (5 per cent of protein) was necessary for normal reproduction.

Recently, workers at the University of

California (M. M. Nelson and H. M. Evans, *J. Nutrition* **51,** *71 (1953)*) have reinvestigated the influence of dietary protein on reproduction and have also made careful observations on the influence of B-vitamins and caloric intake under conditions of low-protein intake.

The basal purified diet had the following percentage composition: alcohol-extracted casein, 24; sucrose, 64; hydrogenated cottonseed oil (Crisco or Primex), 8; and mineral mixture, 4. Crystalline vitamin additions per kilogram of diet were (in milligrams): biotin, 0.3; 2-methyl-1,4-naphthoquinone, 5; thiamine hydrochloride, 5; pyridoxine hydrochloride, 5; pteroylglutamic acid, 5.5; riboflavin, 10; para-aminobenzoic acid, 10; niacin, 20; calcium pantothenate, 50; inositol, 400; and choline chloride, 1000. Each animal received weekly a fat-soluble vitamin mixture (high-potency Sardilene) containing 800 U.S.P. units of vitamin A, 115 chick units of vitamin D, 6 mg. of synthetic DL-alpha-tocopherol, and 650 mg. of corn oil. The casein and sucrose components of the basal mixture were varied so that formulations containing 0, 2.5, 5, 10, 15, 20, and 25 per cent of proteins were available for the feeding trials. The casein contained 83 per cent of crude protein (nitrogen × 6.25).

Normal female rats, 80 to 100 days of age and about 200 g. in body weight, were bred with normal males and placed on the diets on the day of mating, *i.e.*, the day of finding sperm. Ten rats were fed each diet and careful observations were made with respect to weight change during gestation, implantations, fetal resorptions, and average weight of young at birth.

With dietary protein levels in the range of 10 to 25 per cent, all females produced normal litters of 8 to 10 young. Maternal weight gains during gestation, however, decreased from an average of 120 g. on the 25 per cent protein diet to 82 g. on the 10 per cent protein diet. The birth weight of the young also declined (from 6.5 to 5.5 g.) as the dietary protein was decreased from 25 per cent to 10 per cent.

Decreasing the dietary protein to 5 per cent resulted in a maternal weight gain of only 11 g. during gestation. Although implantation occurred normally, 30 per cent of the implantations resulted in resorptions, 17 per cent of the young were born dead, and the young at birth weighed less than 5 g. Doubling the amount of crystalline vitamins and adding crystalline vitamin B_{12} (50 micrograms per kilogram of diet) improved the reproductive performance on the 5 per cent protein diet (no resorptions and only 2 per cent stillborn young).

On the 2.5 per cent level of dietary protein, normal implantations were followed by a 90 per cent incidence of fetal resorption and birth weights averaging 3.4 g. The maternal weight loss during gestation averaged 32 g. Additional crystalline vitamins and vitamin B_{12} again resulted in slight improvement in birth weight of young, better maintenance of maternal weight during gestation, and some reduction in resorptions.

On the protein-free diet, the fetal resorption rate was 90 to 100 per cent, the few young born weighed less than 3 g., and maternal weight loss during gestation averaged 41 g. Extremely rapid resorptions of implantation sites were observed, particularly in the females on 2.5 per cent or protein-free diets. This process was so rapid that when the animals were autopsied at the end of gestation (twenty-one to twenty-three days) no trace of implantation sites could be observed in many of the animals. Added vitamins, including vitamin B_{12}, apparently slowed the resorption process so that more implantation sites were discernible at autopsy.

Undernutrition due to restriction in food intake as a cause of reproductive failure was also investigated by applying the paired feeding technic. Ten female rats (group 1) received the protein-free diet, whereas

another 10 (group 2) were fed the 20 per cent protein diet with double the normal vitamin supplement. Group 2 was limited in food intake to that consumed by group 1. A third group received ad libitum the 20 per cent protein diet. This group consumed an average of 16.5 g. of feed daily per rat and was normal in reproductive performance. On the protein-free diet, 90 per cent of the animals implanted and resorbed with an average feed intake of 9.2 g. (56 per cent of the normal or ad libitum intake). Those paired-fed on the 20 per cent protein diet showed 90 per cent implantations, 10 per cent resorptions, and their young were below normal in birth weight (average, 4.8 g.). The signs of reproductive abnormality observed in the paired-fed animals receiving the 20 per cent protein diet could be corrected by increasing the level of dietary protein to 30 per cent even though the feed intake was limited to the level of consumption on the protein-free diet.

Substitution of the protein-free diet before and during gestation was also investigated. In most animals, only one estrous cycle occurred after the institution of the protein-free diet. Many of the females would not mate under these conditions even when in estrous, as judged by vaginal smears. Those females that mated after three to seven days on the protein-free diet had 100 per cent resorptions in the 80 per cent where implantations had occurred. When the protein-free diet was fed after implantation had occurred (seven days after mating), normal litters were produced in 90 per cent of cases. This, contrasted with the complete reproductive failure when the protein-free diet was fed from mating time, emphasizes the critical need for protein during the early stages of placental and embryonal development.

The effect of a transitory protein deficiency was determined by feeding the protein-free and the 20 per cent protein diet for varying periods. When the protein-free diet was fed for a nine-day period, starting three days after mating, 50 per cent resorptions resulted, whereas only 20 per cent resorptions occurred when the nine-day protein-free feeding was started on the fifth day after mating. These results further emphasize the greater need for dietary protein during the early stages of pregnancy.

No macroscopic congenital abnormalities were observed in any of the young from females receiving the low-protein or protein-free diets. The authors point out that the protein deficiency effects on reproduction show similarities to the effects of pyridoxine deficiency in the rat. It was further noted that the rapidity with which the low-protein and protein-free diets disturbed reproductive function was greater than that observed for any vitamin deficiency imposed upon the female rat in reproduction.

The protein-deficiency studies together with the specific vitamin-deficiency studies (pantothenic acid deficiency, folacin deficiency, pyridoxine deficiency) that have been reported by these workers (M. M. Nelson and H. M. Evans, *J. Nutrition* **31,** *497 (1946)*; **38,** *11 (1949)*; **43,** *281 (1951)*) have added considerably to our knowledge of the importance of adequate diet for normal reproductive function.

AUREOMYCIN AND RIBOFLAVIN METABOLISM IN MICROORGANISMS

Knowledge concerning the mechanism of action of bacteriostatic agents is of importance not only from the standpoint of its value as fundamental information but also because of possible practical application in the therapeutic use of these compounds. It is generally considered that these materials inhibit certain metabolic reactions in the bacterial cell and thus inhibit growth of the microorganism. The classic experiments of

D. D. Woods (*Brit. J. Exp. Path.* **21,** *74 (1940)*) have demonstrated that the sulfa drugs act by inhibiting the utilization of the bacterial metabolite para-aminobenzoic acid. In view of the widespread use of the antibiotics, information concerning their mechanism of action is greatly needed.

J. W. Foster and R. F. Pittillo (*J. Bact.* **66,** *478 (1953)*) have approached this problem with the initial premise that antibiotics act by interference with the production or utilization of bacterial metabolites. They studied the effects of varying the concentration of certain metabolites in the mediums on the inhibitory effect of antibiotics on several bacteria. Agar plates were inoculated with 50 to 150 bacterial cells spread evenly over the surface. Some of the plates contained varying quantities of antibiotics and of various metabolites. The plates were incubated thirty-six hours and then examined for colony formation. The criterion of inhibition was an absence of visible colonies. Foster and Pittillo then measured the concentration of antibiotic required for inhibition as related to the concentration of metabolite being studied. The concentration of antibiotic required for inhibition in the absence of metabolite compared to the concentration required in the presence of the metabolite was called the reversal factor. *Escherichia coli*, strain B, was employed in the initial experiments. However, the significant findings were repeated with *Bacillus mesentericus*, *B. subtilis* var. *niger*, *B. subtilis*, strain H, *B. cereus*, *B. mycoides*, *B. brevis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *B. globigii*, *B. megaterium*, and *Aerobacter aerogenes*. Similar results were obtained with the various species.

In preliminary screening experiments four mixtures of metabolites were employed: casamino acids, a mixture of B-vitamins, a mixture of purines and pyrimidines, and a mixture of ribosides. The antibiotics investigated were aureomycin, chloramphenicol, penicillin G, dihydrostreptomycin and terramycin. Some reversal of the inhibitory effect of each of these antibiotics was produced by one or another of the four mixtures of metabolites employed. The most promising results were obtained with aureomycin and in the subsequent experiments Foster and Pittillo concentrated on this antibiotic.

A study was next made of the reversal effects of various B-vitamins of the inhibition of *E. coli* by aureomycin. Aureomycin concentrations of 0, 0.5, 1, 2, 4, and 8 micrograms per milliliter of agar medium were employed. In the absence of added metabolite 1 microgram of aureomycin was completely inhibitory. Most of the B-vitamins studied exhibited some reversal activity; however, riboflavin was by far the most effective. When riboflavin was added at a concentration of 0.2 micrograms per milliliter, 4 micrograms per milliliter of aureomycin were required for complete inhibition. At a riboflavin concentration of 2 micrograms per milliliter aureomycin exhibited no inhibition at a concentration of 8 micrograms per milliliter, the highest level studied.

The results obtained at this point indicated that aureomycin inhibited bacterial growth because it interfered with the production or utilization of riboflavin. The organisms which had been used do not normally require an exogenous source of riboflavin for growth. For the next experiments a riboflavinless strain of *B. subtilis* was employed. This mutant would grow on the medium used provided riboflavin was added. A study was then made of the optimum concentration of riboflavin for growth of this organism at various concentrations of aureomycin. In the absence of aureomycin 1 microgram per milliliter of riboflavin produced maximum growth. In the presence of 0.5 micrograms per milliliter of aureomycin, 16 micrograms per milliliter of riboflavin were required for maximum growth. The data then indicated a competitive relationship between aureomycin

and riboflavin and suggested that aureomycin inhibited the utilization of riboflavin since the organism studied did not synthesize this vitamin.

The possibility existed that aureomycin also inhibited the synthesis of riboflavin by organisms which do not normally require an exogenous source. To test this hypothesis the organism *A. aerogenes* was cultivated in the presence of 0, 2, and 4 micrograms of aureomycin per milliliter. After twenty-two hours incubation the cells were harvested and assayed for riboflavin by microbiologic assay. Riboflavin synthesized, as micrograms per milligram dry weight of cells, in the presence of 0, 2, and 4 micrograms of aureomycin per milliliter, was 0.0041, 0.0039, and 0.0030 respectively. Thus aureomycin did not markedly inhibit the synthesis of riboflavin.

The results of the experiments suggested that the effectiveness of aureomycin as a bacteriostatic agent should be inversely proportional to the concentration of riboflavin in the cell. It should then be possible to reduce the level of aureomycin required for inhibition by reducing the concentration of riboflavin in the cell. It was found that 1,2-dichloro-4,5-diaminobenzene inhibited riboflavin synthesis in *B. megaterium*. A study was then made of the concentration of aureomycin required for inhibition of *B. subtilis* in the presence of varying amounts of 1,2-dichloro-4,5-diaminobenzene. In the absence of 1,2-dichloro-4,5-diaminobenzene 0.125 micrograms per milliliter of aureomycin were required for complete inhibition, while in the presence of 6 micrograms per milliliter of 1,2-dichloro-4,5-diaminobenzene only 0.0075 micrograms per milliliter of aureomycin completely inhibited the organism. Thus the results supported the concept that the effective inhibitory level of aureomycin varies inversely with the riboflavin content of the cell.

The results of these experiments indicate that aureomycin functions primarily by inhibiting the utilization of riboflavin by bacterial cells. Certain extensions of these findings are indicated. It will be of interest to determine the effects of variations in the dietary intake of riboflavin on the effectiveness of aureomycin in patients. It seems quite possible that as more detailed information concerning the mechanism of action of the various antibiotics is obtained, careful control of the diet will prove to be an extremely valuable adjunct to therapy with these compounds.

NOTES

A British Opinion on Fluoridation

A recent report of a United Kingdom Mission entitled "The Fluoridation of Domestic Water Supplies in North America as a Means of Controlling Dental Caries" (J. R. Forrest, J. Longwell, H. H. Stones, and A. M. Thomson, *Her Majesty's Stationery Office, London (1953)*) presents a clear and realistic view of the fluoridation subject. This review is particularly refreshing by reason of the fact that the four members of the mission were outstanding in one or more phases of dental or scientific pursuits, but yet had not been linked with fluoride studies previously. The report was based on personal observations made by the members of the team during an inspection tour in the United States and Southern Ontario of various fluoride-free areas, natural fluoride areas, and communities where fluorides were artificially introduced into the water supplies. The fifteen appendices contain a concise and valuable compilation of a wide variety of facts pertaining to the fluoride problem. Indeed, at the present time, a comparable tabulation of up-to-date facts about fluorides is not available in the American literature.

The summary and conclusions of this report are particularly forthright. Briefly summarized, they are as follows: (1) Epidemiologic studies in America have demonstrated beyond doubt that among children and adults who have been born and brought up in areas where the drinking water contains fluorides at a level of 1 part per million (p.p.m.) or more, there is much less dental caries than in areas where the drinking water contains little or no fluoride. (2) Evidence among children is conclusive that, in artificial fluoridation areas, there is a reduction in the incidence of dental caries comparable to that experienced where fluorides are a natural component of the water supplies. (3) The possibility of mottled enamel at the 1 p.p.m. level is not a hazard. (4) No scientific evidence exists to indicate a danger to health from the continued consumption of water containing fluorides at the level of 1 p.p.m. (5) Fluoridation of water supplies is preferable to all other methods of administration including topical application of fluoride solutions to tooth surfaces.

The recommendations of this Mission to the Ministry of Health and the Department of Health for Scotland appear to be uniquely fitted to the British needs and briefly summarized are as follows: (1) By reason of the effectiveness of fluoridation in North America it is reasonable to assume that it would be useful in Great Britain. (2) Preliminary typical study centers where the present caries incidence is known should be chosen for testing the effects of artificial fluoridation on medical and dental problems under the existing conditions in these British communities. In particular, it was recommended that a careful assessment be made to determine if different amounts of fluoride should be added to British water supplies than those added in North America, by reason of different food habits, climate, etc. (3) In spite of the fact that the evidence for the harmlessness of fluorides at 1 p.p.m. in the water supplies is strong, they suggested that continued research into the effects on health and disease of fluoride ingestion should be encouraged. (4) About 10 per cent of the population of Great Britian do not obtain their water from a communal supply; hence there is a need to determine how these people may obtain the benefits of fluorides through some other method than fluoridation.

It is noteworthy that the British Mission did not recommend postponement of fluoridation until further research could be done in Great Britain on the effects of fluoride

ingestion on other medical conditions than those in the oral cavity. Their proposal for study centers appeared to be designed primarily to prove the assumption that fluorides would be effective in Great Britain, to check the amount of fluorides needed in Great Britain and the circumstances under which they should be administered. One definite difference between the habits of the population of Great Britain and North America to which attention was drawn was the difference in the tea consumption with the concomitant fluoride ingestion thereby. Another point of contrast was that 90 per cent of the British population consumed water from communal water supplies in contrast to 65 per cent in the United States.

Maize and Maize Diets

The Nutrition Division of the Food and Agriculture Organization has prepared, with the collaboration of Harriette Chick of the Lister Institute of Preventive Medicine, London, a useful survey on corn as a human staple. This little book ("Maize and Maize Diets," *FAO Nutritional Study No. 9, Rome, Italy (1953)*, pp. 94, price: $1.00) deserves to be described briefly for its potential use as an effective teaching aid as well as for its value as a reference document.

An interesting historical introduction retraces the introduction of corn into various parts of the world. A list of documented definitions, stating clearly the exact meaning of a number of terms which have been too often used indiscriminately in describing corn products will doubtless prove valuable to nonspecialists. The morphologic and chemical structures of maize are described from the point of view most likely to interest the nutritionist, with emphasis on the effect of various treatments, storage and milling, on the protein, fat, vitamin and mineral content of corn. Next follows an analysis of the diet of maize eaters which, while excellent, could perhaps have been further expanded for the benefit of readers who do not have at their disposal the documentary material available to the Nutrition Division of FAO. The discussion of pellagra and of the nutritional interrelationship of niacin and tryptophan follow classic lines. The authors have wisely differentiated the improvement of the nutritional value of maize and the improvement of maize diets. There, too, one could wish that detailed descriptions of a number of case studies from different areas had been included. The more than twenty pages of tables represent a wealth of information which would take considerable time and effort to duplicate.

Automatic Recording of Bacterial Growth Rates

Nutritionists interested in studying the growth rates of microorganisms may find the microbial auxanometer described by P. A. Anderson (*J. Gen. Physiol.* **36,** *733 (1953)*) of considerable interest. It is a refinement of the well-known chemostatic methods for the continuous culturing of microorganisms. The culture tube is set up in a turbidimeter which is pre-set to a fixed reference density. If the protoplasmic density in the culture exceeds the reference density, the resulting galvanometer deflection of the turbidimeter activates a relay which opens a magnetic valve and allows fresh nutrient solution to enter the culture tube, thus restoring the original protoplasmic density. Excess liquid is syphoned off from the culture tube, so that volume and density remain essentially constant. The drops of added nutrient solution are counted and automatically recorded. The rate of addition of fresh nutrient solution is thus directly proportional to the growth rate of the culture under study.